マウスにおける塩化メチル水銀の発癌性に及ぼすホルモンの影響

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Hormonal Influence on Carcinogenicity of Methylmercury in Mice

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ABSTRACT. The hormonal influence on the carcinogenicity of methylmercury chloride (MMC) was examined in an 80-week experiment using mice. One hundred intact males, 100 intact females, 100 castrated males, and 100 spayed females were divided into 4 groups: (I) intact mice on a basal diet, (II) intact mice on MMC diet, (III) castrated or spayed mice on MMC diet, and (IV) castrated or spayed mice on MMC diet and testosterone propionate (TP) administered s.c. at 0.2 mg/head/week for the entire period. The dietary concentration of MMC was 10 ppm. The survival rate was lowest in both sexes of Group IV because of the aggravation of the toxic nephropathy and the higher incidence of amyloidosis. The incidence of renal epithelial tumors (15/50) and tubular cell hyperplasia (6/50) in Group II males was significantly increased when compared with the controls. No renal tumors or hyperplastic tubules were induced in Group II females or in either sex of Group III. In Group IV, 2 males and 3 females had renal adenocarcinomas and 3 males and 2 females showed tubular cell hyperplasias, indicating some contributing effects of TP. The present results indicate that the testis may have an important role in the induction of renal tumors in mice by MMC.—KEY WORDS: carcinogenicity, methylmercury, renal tumor, sex difference, testosterone.

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Methylmercury chloride (MMC) is an environmental toxicant which has produced the tragic impairment of the central nervous system known as "Minamata disease". Our previous reports demonstrated that MMC is a carcinogen for the mouse kidney [3, 4]. Our experiments also revealed that the renal tumors were induced only in males, suggesting that some hormonal factors may play a role in MMC tumorigenesis in mice. These results lead us to investigate the effects of castration or spaying and testosterone propionate administration on the carcinogenic potentiality of MMC.

MATERIALS AND METHODS

Methylmercury chloride (MMC) and testosterone propionate (TP) were supplied from Tokyo Kasei Kogyo Co., Ltd., Tokyo. The purity of MMC was 99.3%.

SPF ICR mice (Slc: ICR) of both sexes, raised by Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Shizuoka), were purchased at 4 weeks of age, transferred into a barrier sustained animal room with controlled room temperature at 24±1°C, humidity at 55±5%, and lightening for 14 hrs a day, and housed in groups of 4 animals of the same sex in aluminum cages with wire-mesh floor. Tap water and basal powdered chow, Diet M (Oriental Yeast Co., Ltd., Itabashi, Tokyo) were available *ad libitum*. After a one-week acclimatization period, half the animals were castrated or spayed under Nembutal anesthesia and allowed to recover for 2 weeks. Then, at 7 weeks of age the animals were divided into the following 4 groups each of which consisted of 50 healthy males or females that were either intact or operated: Group I-intact + basal diet, Group II-intact + MMC at 10 ppm in the basal diet (MMC diet), Group III-castrated

or spayed + MMC diet, and Group IV-castrated or spayed + MMC diet + TP injection. TP was administered subcutaneously once a week at a dose of 0.2 mg per mouse in 0.2% suspension (W/V) in sesame oil. The dosage of TP was calculated based on a study in which subcutaneous injections of 1 mg of TP per rat were given to castrated animals [2]. The treatment was continued for a period of 80 weeks.

All the animals were observed daily for their general condition. Each animal was weighed weekly during the first 26 weeks of treatment and biweekly thereafter. Animals found dead or killed in extremis during the study and all survivors after 80 weeks were autopsied. Major organs and tissues from all animals were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned. Sections, 3 or 4 μm in thickness, were stained with hematoxylin and eosin for microscopic observations.

Fisher's exact test was used to compare the frequency of histopathological lesions among the groups. The 5% level of probability was used as the criterion of significance.

RESULTS

Survival rate and body weight: The survival rates in both sexes of Group IV declined after 50 weeks of treatment (Fig. 1). Although Group II also showed lower survival rates in the later period of the study, the rates were much higher than those of Group IV. The major cause of death in the MMC treated animals was nephropathy and/or cecal ulcer.

Both sexes in Group II showed a consistent decrease in body weight gain when compared with the control group (Fig. 2). The body weight changes during MMC treatment in the castrated or spayed animals (Group III) were comparable to or greater than those of the controls. Body weight gain

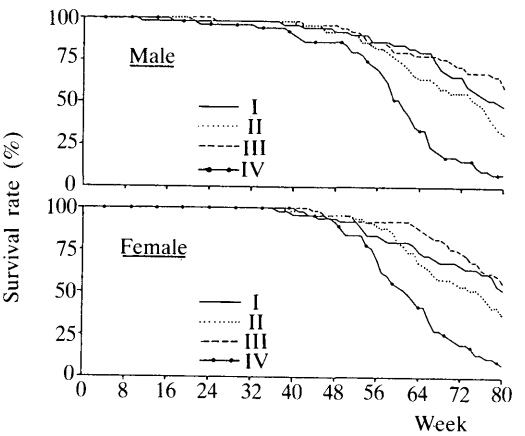

Fig. 1. Survival rate of mice during the study.
—— Group I; intact + basal diet,
····· Group II; intact + MMC at 10 ppm in the basal diet (MMC diet),
------ Group III; castrated or spayed + MMC diet,
—•— Group IV; castrated or spayed + MMC diet +TP s.c. at 0.2 mg/head/week.

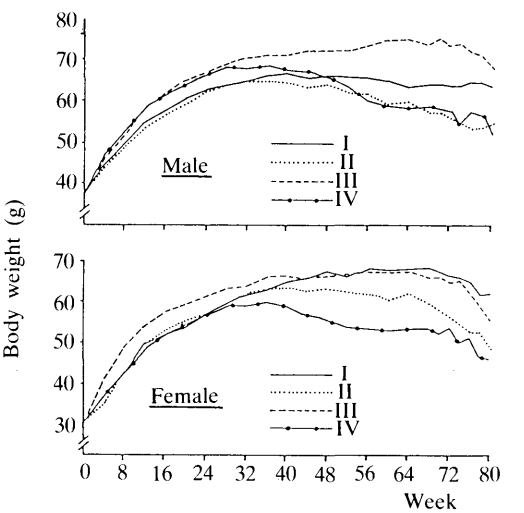

Fig. 2. Mean body weight change of mice during the study.
—— Group I; intact + basal diet,
····· Group II; intact + MMC at 10 ppm in the basal diet (MMC diet),
------ Group III; castrated or spayed + MMC diet,
—•— Group IV; castrated or spayed + MMC diet +TP s.c. at 0.2 mg/head/week.

was more severely depressed in TP-treated females (Group IV) than in Group II females. The body weight in Group IV

males was comparable to that in Group II males.

Treatment-related nonneoplastic lesions (Table 1): Nephrotoxic changes characterized by nuclear swelling and vacuolar degeneration of the cytoplasm of the renal proximal tubular epithelium were seen in both sexes of Groups II, III, and IV killed after 33 weeks. The severity of the toxic changes became more marked in both sexes of Groups II and IV as the treatment was prolonged. Interstitial fibrosis secondary to marked destructive changes of the renal tubular epithelium was frequently encountered, especially in Group II males and in both sexes of Group IV.

The incidence of amyloidosis of the kidney, liver, and spleen was extremely high in both sexes of Group IV when compared with the corresponding control group. In Groups II, III, and IV, cecal ulcers severe enough to penetrate the serosa were seen in both sexes killed after 33 weeks. The incidence of these ulcers in Groups II and IV was 10 of 50 or more, while no males or females in Group I had any cecal ulcers.

Renal tumors (Table 1): The renal tumors observed in the present study were adenocarcinomas except for two adenomas, one occurring in a Group II male and the other in a Group I female. Fourteen adenocarcinomas were detected in Group II males, but no renal tumors were seen in Group II females or in either sex of Group III. In Group IV, adenocarcinomas were seen in 2 males and 3 females, although this incidence was not statistically significant when compared with the control. The adenocarcinomas were usually unilateral and well demarcated from normal tissue. The tumor cells showed solid or papillary proliferation. The solid types showed a pseudoglandular pattern surrounded by delicate stroma, sometimes including glandular luminal spaces (Fig. 3). The papillary types seemed to be growing into large cysts from the lining of epithelial cells (Fig. 4), sometimes including solid type foci and accompanied by hemorrhage and necrotic foci. Although nuclear and cellular pleomorphism of the tumor cells was prominent and mitotic figures were occasionally encountered, metastasis to remote organs was not observed.

Tubular cell hyperplasias regarded as preneoplastic were observed only in Group II males (6/50) and in both sexes of Group

Table 1. Incidence of treatment-related nonneoplastic lesions and renal tumors

	Male				Female			
Group	I	II	III	IV	I	II	III	IV
No. of animals examined	50	50	50	50	50	50	50	50
Kidney:								
Fibrosis	3	32*	9	40*	1	10*	9*	39*
Amyloidosis	22	12	11	34*	6	11	16*	23*
Liver: Amyloidosis	8	10	2	25*	2	8*	9*	29*
Spleen: Amyloidosis	8	10	4	28*	5	5	8	31*
Cecum: Ulcer	0	17*	6*	15*	0	10*	2	18*
Kidney:								
Tublar cell hyperplasia	0	6*	0	3	0	0	0	2
Epithelial tumor	0	15*	0	2	1	0	0	3
Adenoma	0	1	0	0	1	0	0	0
Adenocarcinoma	0	14*	0	2	0	0	0	3

* Significantly different from Group I ($P<0.05$).

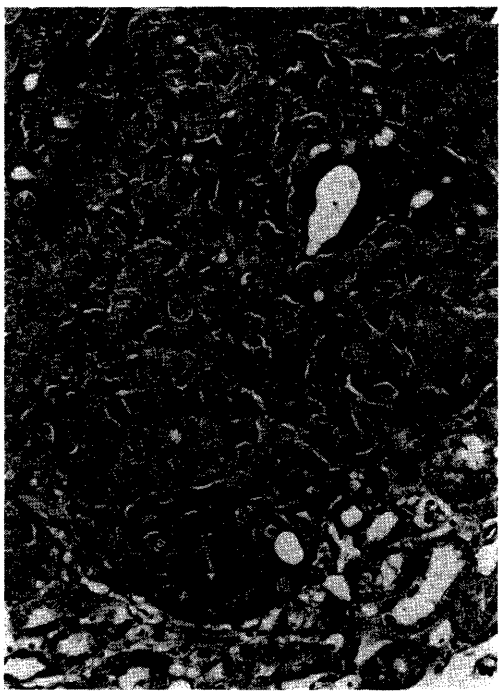

Fig. 3. Solid type of renal adenocarcinoma showing pseudoglandular pattern surrounded by delicate stroma. Nuclear and cellular pleomorphism is prominent and mitotic figures are sometimes observed. Group II male killed by design at 80 weeks. HE stain. ×180.

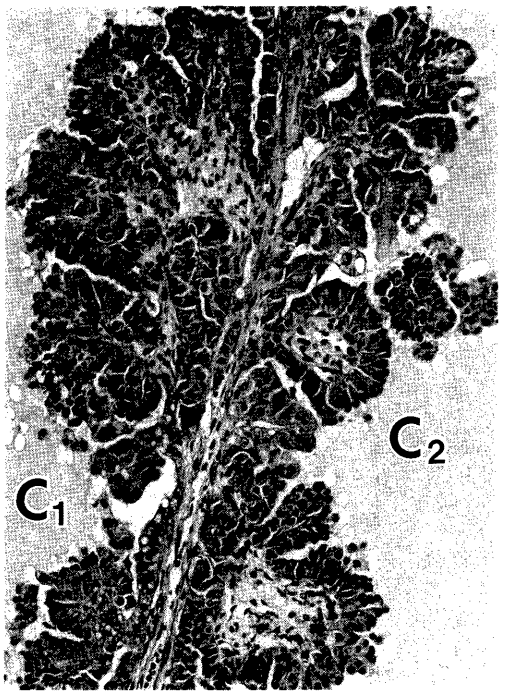

Fig. 4. Papillary type of renal adenocarcinoma showing the growth of the lining epithelial cells into the two cysts (C_1 and C_2). Group II male killed by design at 80 weeks. HE stain. ×156.

IV (3/50 males and 2/50 females). Most of these hyperplastic lesions were characterized by solid growth of large tubular epithelial cells occluding the tubular lumen (Fig. 5). The tumor cells had slightly pleomorphic nuclei and abundant cytoplasm. Some other hyperplastic lesions were cystic tubular hyperplasia consisting of simple papillary growth or stratification of large eosinophilic cells in cystic or dilated tubules (Fig. 6).

Other neoplastic lesions: Neoplastic lesions other than those of the kidney are presented in Table 2 for males and Table 3 for females. The incidence of malignant lymphoma was increased in Group III males when compared with the control. In Group III females the incidence of cortical adenoma of the adrenal was also increased. On the other hand, the incidence of malignant lymphoma in Group IV females and mammary adenocarcinoma in Group III and IV females was depressed. A decreased occurrence of hepatocellular adenoma in Group III and IV males and in Group II and IV females was also noted.

DISCUSSION

It was expected that the TP would masculinize the castrated or spayed animals in Group IV. However, the survival rate of both sexes in this group was much lower than that in Group II, and the body weight gain in Group IV females was severely depressed when compared with that of Group II females. These accelerated toxic manifestations in Group IV were attributed to the aggravation of the toxic nephropathy, because interstitial fibrosis, regarded as the final stage of toxic nephropathy, was more frequently seen in Group IV. Evidence has

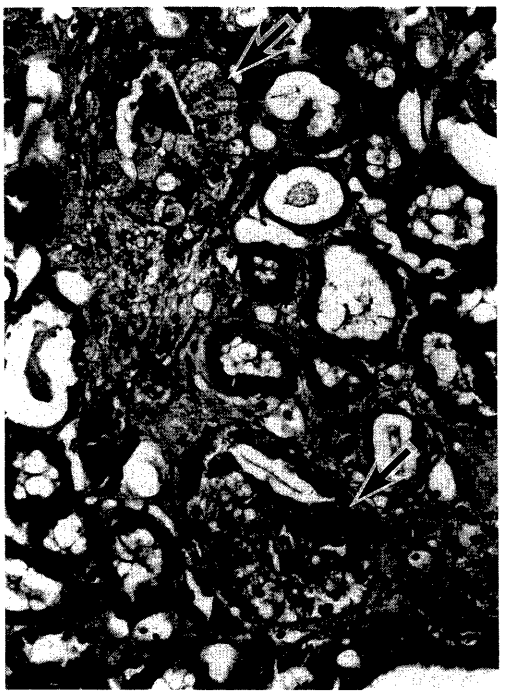

Fig. 5. Two foci (arrows) of tubular cell hyperplasia showing solid growth of large epithelial cells protruding into the lumen. Group II male killed by design at 80 weeks. HE stain. ×195.

Fig. 6. Cystic tubular hyperplasia showing simple papillary growth or stratification of large epithelial cells with slightly pleomorphic nuclei and eosinophilic cytoplasm. Group II male killed by design at 80 weeks. HE stain. ×195.

been accumulating that many nephrotoxicants require metabolic transformation in the kidney and that the severity of the toxic lesions depends on the extent of covalent binding of radical intermediates to macromolecules, which is influenced by the androgenic hormonal status of the animals [6]. The lower survival rate in Group IV might be also related to the higher incidence of amyloidosis of the kidney, liver, and spleen, because systemic amyloidosis is regarded as one of the causes of death in aged mice. However, the cause of the higher incidence of amyloidosis in Group IV was not clarified.

The morphological features of the renal tumors observed in Group II males were comparable to those reported in our previous reports [3, 4]. These renal tumors, induced by MMC in Group II males, were not observed in castrated males (Group III) given the same amount of MMC. This finding suggests that the presence of the testis may have an important role in the induction of renal tumors by MMC. In other words, the carcinogenic potentiality of MMC on the kidney may be influenced by androgenic hormones such as TP. Though 2 males and 3 females of Group IV had renal adenocarcinomas with a morphological resemblance to those observed in Group II males, the incidence of these tumors was not statistically significant when compared with that of the control. However, we can not deny that the occurrence of renal tumors in Group IV is attributable to MMC, since spontaneous renal tumors in mice are extremely rare and their incidence in our historical data on ICR male mice is 0.67% (6/891) and since tubular cell hyperplasia of the renal proximal tubules was observed

Table 2. Incidence of tumors in male mice

Group		I	II	III	IV
No. of animals examined		50	50	50	50
Hematopoietic system					
Systemic:	Malignant lymphoma	1	0	12*	3
	Myelogenic leukemia	1	2	0	0
Thymus:	Malignent thymoma	1	0	0	0
Spleen:	Hemangioma	0	0	1	0
	Hemangiosarcoma	0	2	0	0
Respiratory system					
Lung:	Adenoma	7	6	0*	3
	Adenocarcinoma	6	10	6	3
Digestive system					
Liver:	Hepatocellular adenoma	12	6	4*	2*
	Hepatocellular carcinoma	7	4	7	4
	Hepatoblastoma	2	0	0	0
Genital system					
Prostate:	Leiomyosarcoma	1	0	0	0
Endocrine system					
Pituitary:	Anterior adenocarcinoma	0	0	1	0
Thyroid:	Adenoma	2	0	0	0
Adrenal:	Cortical adenoma	0	0	2	0
	Cortical adenocarcinoma	0	0	1	0
Skin/Subcutis					
	Hemangiosarcoma	0	1	1	0
	Osteosarcoma	1	0	0	0
Harderian gland:	Adenoma	6	8	5	3
	Adenocarcinoma	0	0	2	0
Body cavity					
Abdominal cavity:	Hemangiosarcoma	0	0	1	0

* Significantly different from Group I ($P<0.05$).

only in Group II males and in both sexes of Group IV. A similar tubular cell hyperplasia was observed in rats given gold-containing compounds and was considered as a preneoplastic change for renal tumors [5]. In addition, we must consider the possibility that the lower incidence of renal tumors in Group IV may be due to a decreased number of effective animals after 50 weeks because of the increased number of animals with aggravated toxic nephropathy. It is possible that TP administered once per week enhances the toxic nephropathy. A further study using other dosage and administration methods for TP in which the masculinity of castrated mice resembles normal intact males is needed to clarify the effect of TP on the renal carcinogenicity of MMC.

The change of hormonal status in the animals caused a significant alteration in tumorigenesis. While malignant lymphoma is predominantly observed in females in this strain of mouse [7], a significantly higher incidence was noted in castrated males and a significantly lower occurrence was observed in spayed females administered TP. The incidence of mammary tumors was decreased in the 2 groups of spayed females. The higher rate of cortical adenoma of the adrenal in spayed females was consistent with the results of others [8, 10]. It is also well recognized that nutritional status has a significant influence on tumorigenesis and

Table 3. Incidence of tumors in female mice

Group		I	II	III	IV
No. of animals examined		50	50	50	50
Hematopoietic system					
Systemic:	Malignant lymphoma	9	4	5	2*
	Myelogenic leukemia	2	0	0	1
Thymus:	Thymoma	0	0	1	0
Spleen:	Hemangioma	0	0	1	0
Lymph node:	Histiocytic sarcoma	0	0	1	0
Respiratory system					
Lung:	Adenoma	3	1	4	3
	Adenocarcinoma	5	7	9	3
Digestive system					
Forestomach:	Papilloma	0	1	0	0
	Squamous cell carcinoma	0	0	1	0
Liver:	Hepatocellular adenoma	5	0*	8	0*
	Hemangioma	0	1	0	0
	Hepatocellular carcinoma	1	0	3	0
Genital system					
Ovary:	Adenoma	2	0	0	0
Uterus:	Adenoma	0	0	0	1
	Leiomyoma	1	2	0	1
	Hemangioma	0	1	0	0
	Adenocarcinoma	0	0	1	0
Endocrine system					
Pituitary:	Anterior adenoma	1	0	2	0
Adrenal:	Cortical adenoma	0	0	7*	3
	Pheochromocytoma	0	1	3	0
	Cortical adenocarcinoma	0	1	3	0
Nervous system					
Cerebrum:	Meningioma	0	0	1	0
Skin/Subcutis					
	Papilloma	0	1	0	0
	Basal cell carcinoma	0	1	0	0
	Squamous cell carcinoma	0	0	1	0
	Hemangioma	0	0	1	0
	Rhabdomyosarcoma	1	0	0	0
	Hemangiosarcoma	0	0	1	0
Harderian gland:	Adenoma	6	3	5	3
Mammary gland:	Adenoma	0	2	0	0
	Adenocarcinoma	18	12	0*	0*

* Significantly different from Group I ($P<0.05$).

undernutrition is associated with a lower occurrence of spontaneous tumors [1, 9].This may be reflected in the lower incidence of hepatocellular adenoma in castrated males administered TP, in intact females given the MMC diet, and in spayed females administered TP.

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要　約

マウスにおける塩化メチル水銀の発癌性に及ぼすホルモンの影響：平野雅裕・上田英夫・三森国敏・真板敬三・白須泰彦(残留農薬研究所毒性部)——塩化メチル水銀のマウスの腎発癌性に及ぼすホルモンの影響を以下の4群の雌雄動物(各群各性50匹)について検索した;Ⅰ群—無処置，基礎飼料給餌群，Ⅱ群—無処置，塩化メチル水銀10ppm 添加飼料群(MMC 飼料)，Ⅲ群—精巣(卵巣)摘出，MMC 飼料群，Ⅳ群—精巣(卵巣)摘出，テストステロンプロピオネイト(TP)s.c. 0.2mg/頭/週，MMC 飼料群．生存率は雌雄ともⅣ群において最も低く，これは，中毒性腎障害の重篤化およびアミロイドーシスの発生頻度増加に起因するものであった．腎上皮性腫瘍(15/50)および尿細管上皮過形成(6/50)の発生頻度はⅡ群雄において有意に増加した．Ⅱ群雌およびⅢ群の雌雄においては，腎腫瘍および上皮過形成の発現は認められなかった．Ⅳ群では，腎腺癌が雄2匹，雌3匹に，尿細管上皮過形成が雄3匹，雌2匹に観察され，TP の関与が示唆された．以上の結果から，塩化メチル水銀によるマウスでの腎腫瘍誘発には精巣の存在が重要であると推察された．